Evolutionary history expands the range of signaling interactions in hybrid multikinase networks

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Published in:
Scientific Reports

DOI:
10.1038/s41598-021-91260-w

Publication date:
2021

Citation for published version (APA):
Ortet, P., Fochesato, S., Bitbol, A-F., Whitworth, D. E., Lalaouna, D., Santaella, C., Heulin, T., Achouak, W., & Barakat, M. (2021). Evolutionary history expands the range of signaling interactions in hybrid multikinase networks. *Scientific Reports*, *11*(1), 11763. [11763]. https://doi.org/10.1038/s41598-021-91260-w

Supplementary Information

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TABLE S1: Strains and plasmids used in this study

Strain or plasmid	Description	Reference
Strains		
Escherichia coli		
TOP10	F− mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(Strr) endA1 λ−	Invitrogen
GM2163	F− dam-13::Tn9 dcm-6 hsdR2 leuB6 his-4 thi-1 ara-14 lacY1 galK2 galT22 xyl-5 mtl-1 rpsL136 tonA31 tsx-78 supE44 McrA− McrB−	
DH5alpha	F- 80dlacZ M15 (lacZYA-argF) U169 recA1 endA1hsdR17(rk-, mk+) phoAsupE44 -thi-1 gyrA96 relA1	
DH5alpha pRK2013	tra+ , ina- , Kanr , Amps , lux-	
Pseudomonas brassicacearum		
NFM421	Wild type	Ref. 27
NFM421 gacS	gacS mutant	This study
NFM421 gacA	gacA mutant	Ref. 23
NFM421 Psebr_a3082	Psebr_a3082 mutant	This study
NFM421 gacS - Psebr_a3082	gacS - Psebr_a3082 double mutant	This study
Plasmids		
Overexpression		
pME6032	NruI-EcoRI lacIq-Ptac fragment of pJF118EH subcloned in [BamHI]-EcoRI-digested pME6031; lacIq-Ptac expression vector	Ref. 46
pME6032-gacS	pME6032 derivate containing gacS	This study
pME6032-chimera1	pME6032 derivate containing gacS-Psebr_a625_1	This study
pME6032-chimera2	pME6032 derivate containing gacS-Psebr_a625_2	This study
pME6032-chimera3	pME6032 derivate containing gacS-Psebr_a1408	This study
pME6032-chimera4	pME6032 derivate containing gacS-Psebr_a1633	This study
pME6032-chimera5	pME6032 derivate containing gacS-Psebr_a4938	This study
pME6032-chimera6	pME6032 derivate containing gacS-Psebr_a4122	This study
pME6032-chimera7	pME6032 derivate containing gacS-Psebr_a2650	This study
pME6032-chimera8	pME6032 derivate containing gacS-Psebr_a3082	This study
pME6032-chimera8.1	pME6032 derivate containing gacSH294A-Psebr_a3082_3-	This study
pME6032-chimera8.2	pME6032 derivate containing gacS-Psebr_a3082_3-D718A	This study
pME6032-chimera9	pME6032 derivate containing gacS-Psebr_a3692	This study
Mutagenesis		
pCM184	DH5alpha-based suicide plasmid, Kmr	Ref. 45
pCM184-Psebr_a3082::Kmr	pME3087 derivate containing a 788 nucleotides fragment of Psebr_a3082 gene	This study
Insertion in Psebr_a3082	DNA fragment size: 788 nucleotides at positions 1190 - 1977	This study

TABLE S2: Primers used in mutagenesis and qRT-PCR analysis (5’ to 3’ orientation)

Gene	Primer	Sequence
Mutagenesis		
Psebr_a3082	F1189	AGGAAGTGCTGGCCGAAAC
	R1977	TTCGAAGATGCTTTGCTGCTG
gacS	gacS1E	CATAGAATTCCCATGTCGATGATGCGGTCCAC
	gacS2	GAATCGATACTTGTCTCCTGCATCCAGCGTCTG
	gacS3	GTATCGATTCCAGGGCAAGATCCAGGGAAC
	gacS4B	CATAGGATCCGTTGGGGAAGGTCAACAGCC
qRT-PCR		
rRNA 16S	16S-F	CGGAATTACTGGGCGTAAAGC
	16S-R	CAGTGTCAGTATCAGTCCAGG
	TaqMan-rRNA 16S	CTCAACCTGGGAACTGCATTCAAAACTGTC
rsmX	rsmX-F	GTTCTGCAGTCCACTGAAGCACAGGAAGT
	rsmX-R	GACCATTACGACTCCCTGTC
	TaqMan-rsmX	CAGGATCAGGGACGATCGACCTTGC

FIGURE S1

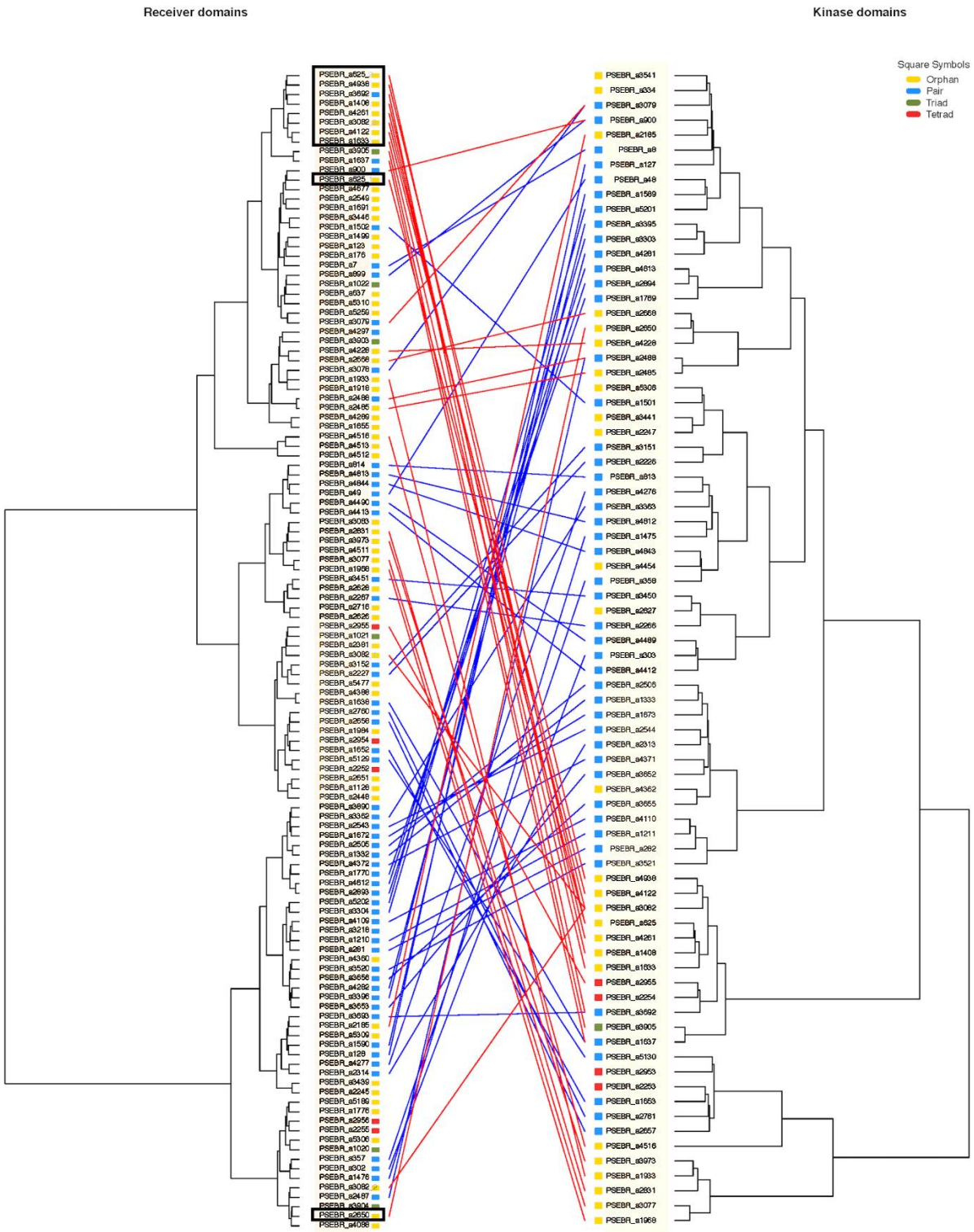
Receiver domains
Kinase domains
Square Symbols
Orphan
Pair
Triad
Tetrad

FIGURE S1. **Paired phylogenetic trees from *Pseudomonas brassicacearum***

Blue lines join paired genes. Red lines connect hybrid or unorthodox HKs to their internal receivers. Blue boxes indicate paired genes, while yellow boxes denote orphan genes. Green and red boxes indicate that the gene belongs respectively to a locus containing three (triad) and four (tetrad) TCS genes. Black rectangles highlight the receiver domains of hybrid HKs used in our study. The paired trees are present in the p2cs database as 'Partnership View' functionality. The database also contains information on the topology of each protein and displays tools, which allow the user to determine on the fly the topology of each HK.

FIGURE S2

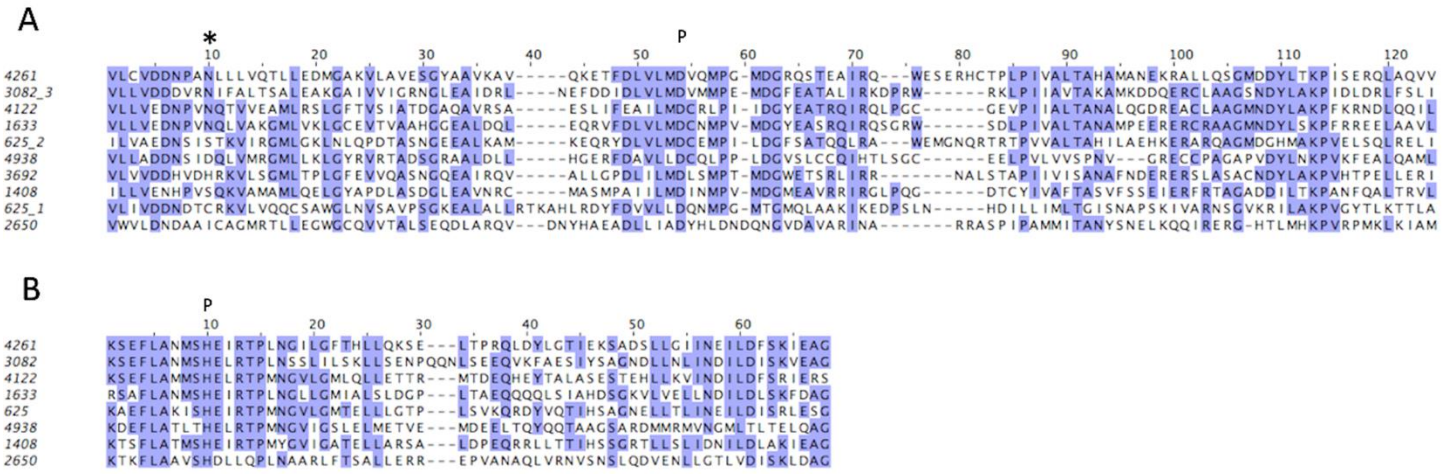

FIGURE S2 Multiple sequence alignment of the receiver and kinase domains

The receiver (A) and kinase (B) domains of the HKs belonging to GacS cluster and the two outsiders are aligned using MAFFT. Amino acid residues are numbered according to their position in the domain. Residues highly conserved (at a threshold of 40%) across all receiver and kinase domains are shaded in purple. (*) Conserved residues in GacS and the three phylogenetically closest HKs (3082, 4122 and 1633), (P) phosphorylation sites. The output view is visualized using Jalview.

FIGURE S3

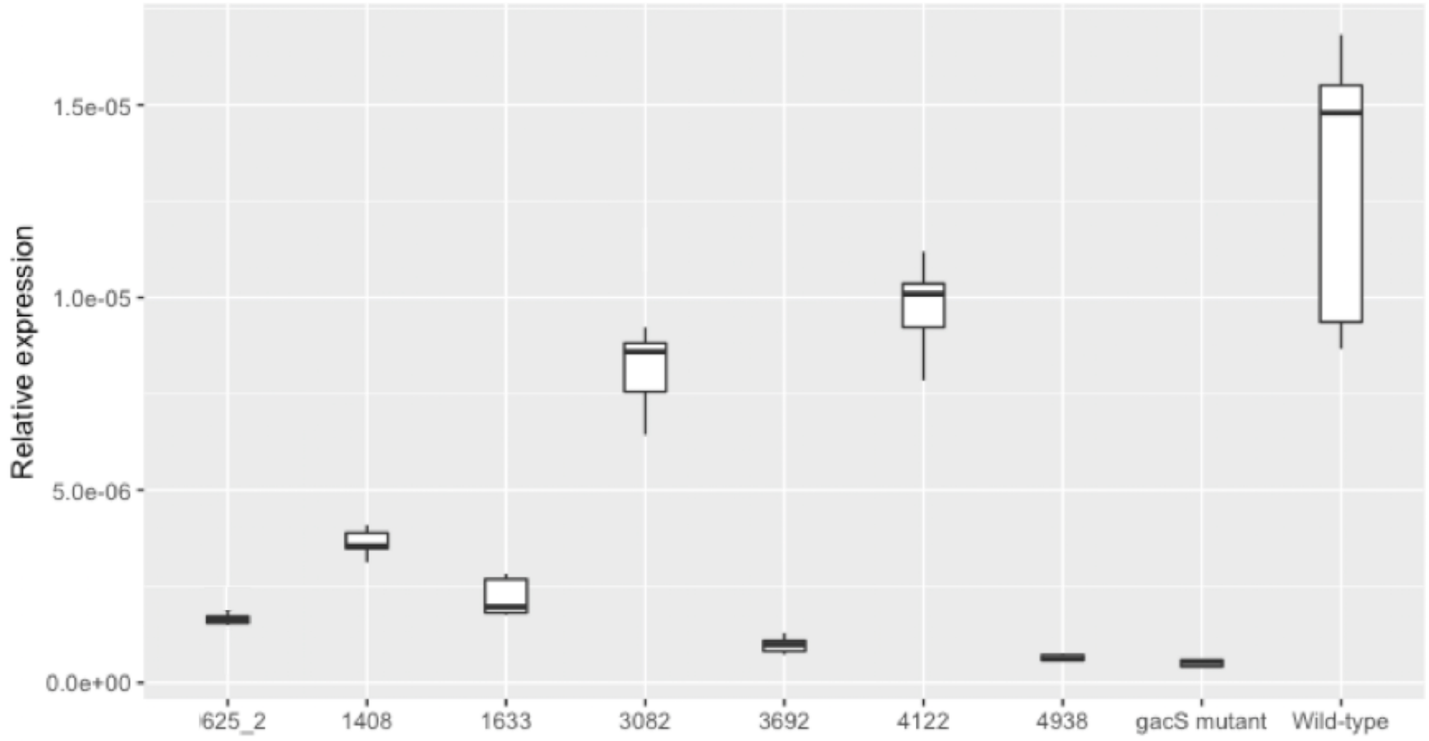

FIGURE S3. **Boxplots of the Welch's heteroscedastic F test**

The test was performed on the *rsmX* gene expression data, using the R package 'onewaytests'. The result of the statistical test is reported in Figure 4.

FIGURE S4

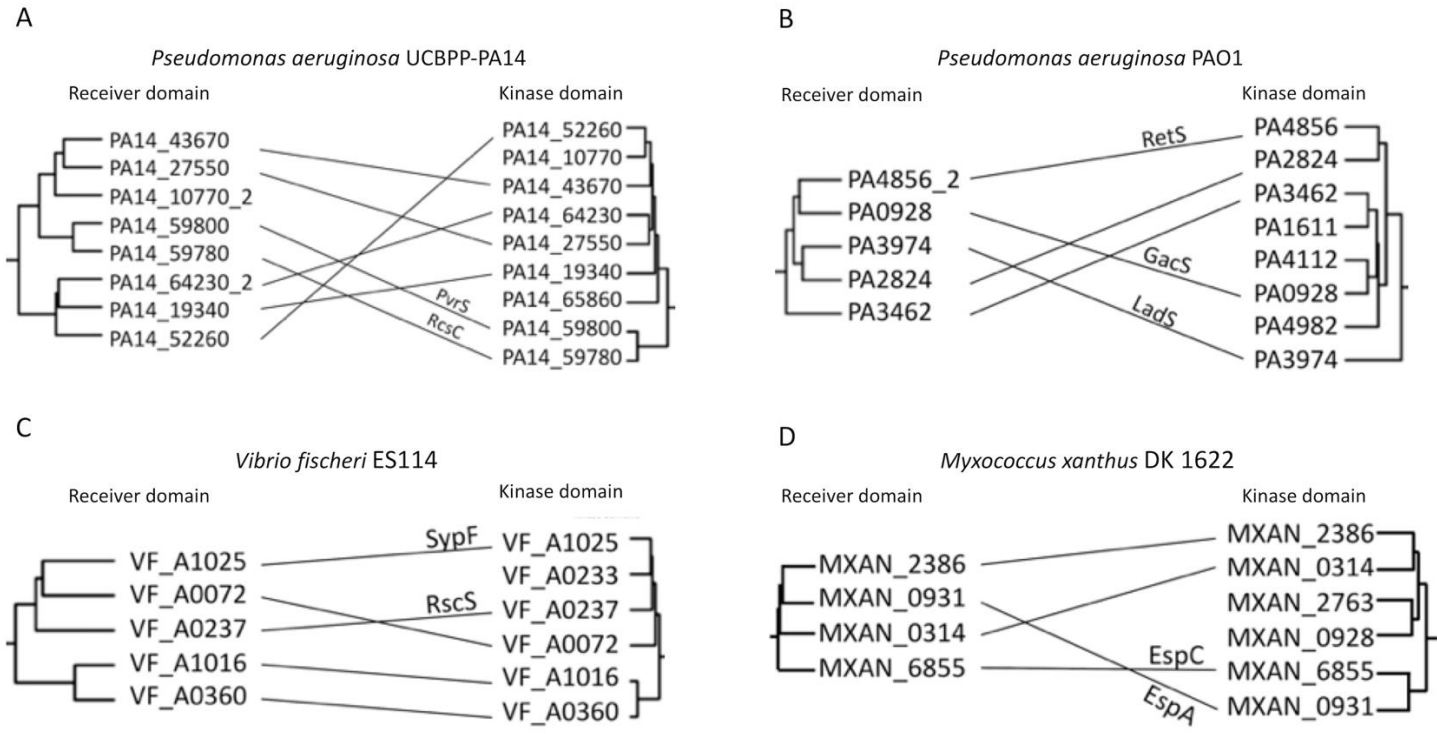

FIGURE S4. **Paired phylogenetic trees**

Pseudomonas aeruginosa PA14, focus on the 'PvrS-RcsC cluster' (A). *Pseudomonas aeruginosa* PAO1, focus on the 'GacS cluster' (B). *Vibrio fischeri* ES114, focus on the 'SypF-RscS cluster' (C). *Myxococcus xanthus* DK 1622. focus on the 'EspA-EspC cluster' (D). Black lines connect hybrid HKs to their internal receivers.